Fungicides Added to Potting Substrate Affect Mycorrhizal Symbiosis between a Peach–Almond Rootstock and *Glomus* sp.

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Additional index words. arbuscular mycorrhiza, esterase activity, glomales, metalaxyl, nontarget organisms, propamocarb, peach–almond rootstock

Abstract. Prior to the commercial use of arbuscular mycorrhiza (AM) in the nursery, the effects of commonly used pesticides on symbiosis must be evaluated. Metalaxyl and propamocarb are two fungicides added to potting substrates to prevent diseases caused by phycomycetes. Both fungicides were incorporated into the potting substrate before the inoculation and planting of the peach–almond hybrid rootstock GF677 (*Prunus persica* L. x *Prunus dulcis* Mill. D.A. Webb). The AM fungus used were *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe and *Glomus intraradices* Schenck & Smith. *Glomus intraradices* was not affected by either fungicide; however, metalaxyl adversely affected root colonization by *G. mosseae* and decreased rhizosphere activity as measured by esterase activity. Chemical names used: *N*-(2-methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate (metalaxyl); propyl 3(dimethylamino)propylcarbamate (propamocarb).

In the Mediterranean countries, many semi-arid zones are increasingly threatened by desertification and soil loss. In these areas, the use of arbuscular mycorrhiza (AM) in revegetation and in agriculture has clear benefits, both for increasing plant growth, especially of long-term crops, such as citrus and fruit trees, and as a step toward reestablishing a more sustainable system that preserves a life-supporting soil matrix.

However, typical nurseries do not produce mycorrhizal plants. This is especially true for micropropagated plants, which are produced under highly artificial conditions and released to the grower devoid of mycorrhizae and other beneficial microorganisms of the rhizosphere. As the awareness of the role of AM symbiosis increases, inoculation with AM fungi will be integrated into nursery management. Before this occurs, AM fungi must be evaluated to select those isolates that are more effective and also more adapted to some of the horticultural practices implemented at the nursery level.

Fungicides are used in most nurseries that are technologically advanced and are using micropropagated rootstocks. Fungicides act upon a great number of organisms for which the chemicals were not specifically formulated. This has resulted in altered biological phenomena that can be favorable or unfavorable for the plant's physiology, for the patho-

gen activity and for disease development (Rodríguez-Kábana and Curl, 1980). Pesticides may influence the biological activity of a soil, which is the result of microbial and enzymatic transformations. The esterase activity of the substrate is a measure of this activity (Schnurer and Rosswald, 1982) and has been used to evaluate changes in microbial respiration (Camprubí et al., 1995; Inbar et al., 1991).

Mycorrhizal fungi play an important role in the soil ecosystem, acting as a link between the autotrophic host plant and the saprophytic decomposers (Jeffries and Barea, 1994). Any compounds that affect the host plant or the soil microflora, such as herbicides, fungicides, and broad spectrum biocides, are bound to affect the mycorrhizal fungi. Effective biocides, such as methyl bromide, methylisothiocyanate, and formaldehyde, have a deleterious effect on the AM symbiosis. Investigations on the effects of fungicides have yielded contradictory results: both systemic and nonsystemic fungicides can reduce (Bailey and Safir, 1978; Fitter, 1986; Habte and Majunath, 1992; Ocampo and Hayman, 1980; Sukarno et al., 1993;), have no effect (Nemec, 1980; Ocampo and Hayman, 1980;), or even increase (Jabaji-Hare and Kendrick, 1987; Land et al., 1993; von Alten et al., 1993) AM root colonization.

Factors that may be responsible for this include:

a) Chemical properties of the compound dictate uptake by the host plant, the storage site (in the soil or in the plant), and the biological processes affected.

b) The pesticide application may be too late; AM root colonization, if already established, may not be affected (Rhodes and Larsen 1981; Sukarno et al. 1993).

c) Host plant species respond differently to

chemical compounds. These responses will in turn affect the fungus.

d) Different AM fungi can respond differently to the same compound.

Our aim was to assess the effects of two different systemic fungicides, metalaxyl and propamocarb, commonly used as soil drenches to prevent soilborne fungal diseases in nursery operations, on two species of AM fungi: *Glomus mosseae* and *G. intraradices*. These fungi promote citrus tree growth in the field (Camprubí, 1994) and could be used in the future as inoculants in commercial nurseries for micropropagated *Prunus* rootstocks (Estaún et al., 1993).

Materials and Methods

Plant material. The peach–almond hybrid rootstock GF677 was used as the host plant. This hybrid is the most popular rootstock for peaches in Spain and Southern Europe because of its excellent vigor, good compatibility with peach varieties, adaptation to dry climatic conditions and resistance to chlorosis (Carrera, 1992). The plants were micropropagated and were obtained from a commercial firm (Agromillora Catalana, St. Sadurní d'Anoia, Barcelona) following acclimation.

Arbuscular mycorrhiza inoculum. The inoculum was a mixture of soil and roots of *Allium porrum* L. plants grown on sandy soil and colonized by *G. mosseae* (Nicol. & Gerd.) Gerdemann & Trappe (eight sporocarps/g soil) or *G. intraradices* Schenck & Smith (>50 spores/g soil). Each plant was inoculated with 10 g of the appropriate inoculum placed under the root system at the moment of planting.

Potting substrate. The substrate (2 L/pot) was a mixture of equal volumes of a commercial compost BVU (Prodeasa Ltd., Vilablleix, Girona, Spain) and quartz sand with a pH of 7.3, N (Kjeldahl) content of 0.9 g·kg⁻¹, and P (Olsen) content of 0.1 g·kg⁻¹.

Fungicides. The fungicides tested were propamocarb and metalaxyl. Both fungicides are used in commercial nurseries, and are incorporated in the potting substrates as a standard preventive treatment against diseases caused by the phycomycetes *Phytophthora* sp. and *Pythium* sp.

The doses used in the experiment were the recommended for nurseries. Propamocarb (Previcur, Schering AG) is a carbamate with systemic properties and is effective against phycomycetes. It is used as a soil-applied fungicide but is also suitable as a seed treatment. The dose used was 350 mL·m⁻³. Metalaxyl (Ridomil, Ciba Geigy) is a phenylamide with systemic properties and is applied as a foliar treatment against phycomycetes and also as a soil treatment. The dose used was 20 g·m⁻².

Experimental design. The experimental design was a 3 × 3 factorial with 10 plants per treatment. The experimental groups resulted from the combination of two factors: 1) inoculation treatments were: control, *G. mosseae*, and *G. intraradices*, and 2) fungicide treatments were: control, propamocarb, and metalaxyl. The fungicides were applied to the

Received for publication 20 Oct. 1996. Accepted for publication 1 June 1998. This work was partially supported by the Comisión Interministerial de Ciencia y Tecnología (CICYT) grant AGF92-0428. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

moist substrate the day before transplanting and inoculating. Plants were grown under greenhouse conditions. There were two harvest dates, 8 and 12 weeks after transplant, with five plants per treatment and per harvest. Levels of significance of main treatments and their interactions were calculated by analysis of variance and means were compared by LSD_{0.01}.

Parameters studied. Plant growth was measured as height, dry weight, and stem diameter. Colonization by AM was recorded after staining (Koske and Gemma, 1989) with the gridline intersect method (Giovannetti and Mosse, 1980). Esterase activity, as a measure of substrate respiration and subsequently of microbiological activity, was determined by fluorescein diacetate (FDA) hydrolysis (Camprubí et al., 1995; Inbar et al., 1991). Schnurer and Rosswald, 1982) using a Shimadzu UV-260 spectrophotometer (Shimadzu Corp., Kyoto, Japan).

Results and Discussion

Plant height and root : shoot ratio were significantly affected by treatment (Table 1), but other phenologic parameters measured were not. Therefore, only significant effects are discussed.

The two AM fungi differed substantially in their aptitudes for root colonization and in their effects on plant growth. When considering the height and the root/shoot ratio (R/S) at the first harvest, both parameters measured showed interactions between inoculation and fungicide treatments; therefore, all nine treatments could not be compared and results are discussed according to common factors.

To evaluate the effect of the fungicide on plant height, treatments with no mycorrhiza were examined. Metalaxyl had little effect, but propamocarb stimulated growth compared with the noninoculated fungicide-free control. The effects of the inoculation can be observed by comparing the treatments with no fungicide. At the first harvest, plants inoculated with *G. mosseae* had a low level of root colonization (<20%) and their growth was suppressed. By the second harvest, the levels of colonization had increased, but the effects on plant growth remained the same. Larsen et al. (1996) attributed major suppression of growth in cucumber (*Cucumis sativus* L.) plants inoculated with *G. caledonium* (Nicol. & Gerd.) Trappe and Gerdeman to the presence of growth-inhibiting microorganisms. Minor growth reductions for the same *Glomus*-cucumber symbiosis have been attributed to a fungal carbon drain in high root density systems (Ravnskov and Jakobsen, 1995). In our experiments the characteristics of the minor reduction observed (independence from the percentage root colonized and prevention of the effects with the pesticide treatment) can be attributed to the presence of unidentified growth inhibiting microorganisms. *Glomus intraradices* was a better root colonizer than was *G. mosseae*, with 59% to 79% colonization within 8 weeks (Table 1). Mycorrhizal status did not affect plant height, because the

high nutrient levels of the potting media masked the effect of symbiosis in stimulating plant growth. However, the root/shoot ratio (R/S), which is negatively correlated with the efficiency of symbiosis (Harley and Smith, 1983; Schubert and Hayman, 1986), was smaller in the plants inoculated with *G. intraradices* that were not treated with fungicides. Treatment with propamocarb stimulated the growth of both noninoculated plants and plants inoculated with *G. mosseae* at both harvests (Table 1). Twelve weeks after the application of fungicides, plants treated with metalaxyl were significantly less colonized by *G. mosseae* than either the controls or those that had been treated with propamocarb. At both harvests, plants both inoculated with *G. intraradices* and treated with propamocarb showed the highest root colonization level and achieved a lower R/S.

Enzyme activity. Six days after fungicide application and AM inoculation esterase activity was significantly higher in all plants treated with metalaxyl (Table 2). After 12

weeks, esterase activity had decreased in the metalaxyl-treated plants and had reached values that were significantly lower than in the controls and in the propamocarb treated plants.

Metalaxyl and propamocarb are both systemic fungicides that disrupt fungal cell division. Metalaxyl inhibits RNA synthesis and propamocarb affects cell wall growth (Corbett et al., 1984). They affect AM fungi differently than do the rest of the soil microflora.

Metalaxyl had a stimulatory effect on esterase activity that decreased with time. This transient increase in esterase activity could be the result of an enrichment of the pool of available nutrients following the suppression of certain soil microorganisms by the fungicide, or to an alteration of the chemical properties of the enzyme by the pesticide that reverts with time (Gianfreda et al., 1995). In contrast, propamocarb had no effect on the soil esterase activity.

Although neither of the fungicides affected initial root colonization, *G. intraradices* and *G. mosseae* colonization was reduced by

Table 1. Effects of inoculation with arbuscular mycorrhiza (AM) fungi and fungicide treatment on height, root : shoot ratio (R : S), and arbuscular-mycorrhizal root colonization of the *Prunus* rootstock GF677.

Treatments		1st harvest (8 wk)			2nd harvest (12 wk)		
		Height (cm)	R/S ratio	% Colonization	Height (cm)	R : S ratio	% Colonization
None	Control	24.9	0.86	NS	27.6	0.92	NS
	Metalaxyl	23.9	0.62	NS	23.2	0.91	NS
	Propamocarb	31.0	0.57	NS	33.0	0.67	NS
GM ¹	Control	17.3	0.75	13.8	18.0	1.12	56.6
	Metalaxyl	20.1	0.62	12.4	19.2	1.25	39.8
	Propamocarb	31.5	0.48	17.0	32	0.78	50.4
GI ²	Control	25.9	0.46	57.0	27.2	0.85	84.6
	Metalaxyl	22.4	0.62	70.0	23.0	0.94	75.3
	Propamocarb	27.6	0.51	79.0	26.0	0.77	91.0
LSD _{0.01}		3.62	0.07	9.28	4.22	0.09	6.41

Analysis of variance

Mycorrhiza (M)	*	*	***	*	*	***
Fungicide (F)	***	*	NS	***	***	*
M × F	***	*	NS	**	NS	NS

¹Inoculated with *Glomus mosseae* (GM) or with *G. intraradices* (GI).

NS, *, **, *** Nonsignificant or significant at $P = 0.05, 0.01, \text{ or } 0.001$, respectively, by ANOVA.

Table 2. Effects of inoculation with arbuscular mycorrhiza (AM) fungi and fungicide treatment on the esterase activity of the rhizosphere of the *Prunus* rootstock GF677.

Treatments		Activity ($\times 10^{-4}$ mg fluorescein/min/g substrate)			
		Time after treatment (d)			
Mycorrhiza	Fungicide	0	6	56	84
None	Control	5.09	2.07	0.66	1.41
	Metalaxyl	9.81	5.28	0.63	1.04
	Propamocarb	4.33	1.52	0.73	1.49
GM ¹	Control	---	0.89	0.60	1.25
	Metalaxyl	---	4.60	0.75	0.90
	Propamocarb	---	1.08	0.53	1.09
GI ²	Control	---	1.37	0.64	1.25
	Metalaxyl	---	6.02	0.50	1.00
	Propamocarb	---	1.77	0.61	1.21
LSD _{0.01}		3.21	1.58	0.24	0.39

Analysis of variance

Mycorrhiza (M)	**	NS	**
Fungicide (F)	***	NS	***
M × F	**	NS	NS

¹Inoculated with *Glomus mosseae* (GM) or with *G. intraradices* (GI).

NS, *, **, *** Nonsignificant or significant at $P = 0.05, 0.01, \text{ or } 0.001$, respectively, by ANOVA.

metalaxyl, which stimulates AM development and formation in citrus trees inoculated with *G. etunicatum* Becker & Gerdeman (Nemec, 1980) and in *Allium cepa* L., *Gossypium hirsutum* L. and *Capsicum annuum* L. inoculated with *G. intraradices* (Affek et al., 1991). However, Sukarno et al. (1993) found that metalaxyl reduced both colonization and spore production of *Glomus* sp. in *Allium cepa* roots. No information is available on the effects of propamocarb on AM, although other related compounds, such as prothiocarb {S-ethyl[3-(dimethylamino)propyl] carbamothioate}, inhibit the symbiosis (Trappe et al., 1984). In our study, propamocarb stimulated root colonization by *G. intraradices* and promoted plant growth.

Discrepancies between our data and the results of previous research can be attributed to differences in methodology, AM fungus, and host plant (Affek et al., 1991; Arens et al., 1981; Groth and Martinson, 1983; Nemec, 1980; Sukarno et al., 1993; Trappe et al., 1984). These discrepancies are compounded by differences in the biochemical mechanisms of action of the fungicides involved, and in their effects on rhizosphere microorganisms. Benomyl [methyl 1-(butylcarbamoyl)benzimidazol-2-ylcarbamate] can suppress AM colonization (Sukarno et al., 1993; Trappe et al., 1984). It inhibits DNA synthesis, and, like metalaxyl and propamocarb, disrupts fungal cell division; however, benomyl accumulates in roots and may be taken up easily by the AM fungus growing in root tissues. Neither metalaxyl nor propamocarb accumulate in the root tissues, so their primary effect on the AM symbiosis must be directly on the fungi growing outside the root. Affek et al. (1991) found that metalaxyl increases AM colonization by *G. intraradices* when the soils have not been fumigated. Metalaxyl can affect other soil microflora; thus, the increase in growth shown by Affek et al. (1991) could be due to a decrease in the number of soil microorganisms that negatively affect the AM fungus (Bagyaraj, 1984).

In intensive agriculture and horticulture, disease control programs involve several fungicides. The careful selection of these fungicides might allow the use of AM and other beneficial soil microbiota to improve crop production.

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